APPENDIX E

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Isolated Tumor Cells Are Frequently Detectable in the Peritoneal Cavity of Gastric and Colorectal Cancer Patients and Serve as a New Prognostic Marker

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Objective
To evaluate the prognostic significance of isolated tumor cells detected by a panel of various monoclonal antibodies.

Summary Background Data
Previously, we showed by using immunocytology that cancer cells are frequently found in bone marrow and peritoneal cavity samples of gastrointestinal cancer patients.

Methods
Findings in bone marrow and peritoneal cavity samples were compared and correlated with the 4-year survival rate of 84 gastric and 109 colorectal patients with cancer.

Results
Although positive results in the bone marrow showed little prognostic significance, the peritoneal cavity results correlated with the 4-year survival rate (gastric cancer: $p = 0.0038$; colorectal cancer: $p = 0.0079$). Additionally, in subgroups of patients with early (gastric cancer: $p = 0.02$, colorectal cancer: $p = 0.48$) and advanced (gastric cancer: $p = 0.02$, colorectal cancer: $p < 0.0001$) tumor stages, a correlation of immunocytologic findings and the survival rate was seen.

Conclusions
The detection of minimal residual disease in the peritoneal cavity serves as a new prognostic marker.

The success of surgical treatment in patients with gastric and colorectal cancer is often limited. This is because of local recurrence or the development of distant metastases or peritoneal carcinosis by cells that have already been seeded at the time of operation but cannot be detected using conventional diagnostic tools. The elimination of these micrometastatic cells is the aim of various adjuvant therapies,[1,2] and obviously it would be helpful to detect minimal residual disease.

Using immunocytologic methods, which are significantly more sensitive than conventional cytology,[3] it has become possible to detect disseminated tumor cells in the bone marrow of patients with breast cancer,[4] small cell lung cancer,[5] neuroblastoma,[6] prostatic cancer,[7] gastric cancer,[8] colorectal cancer,[9] and pancreatic cancer.[10]

Bone marrow metastases are rare in gastric and colorectal cancer.[11] The high frequency of intraperitoneal tumor relapse and peritoneal carcinosis strongly suggests that micrometastatic cells are most likely present within the peritoneal cavity.

Previously, we showed that disseminated cancer cells become specifically detectable in the peritoneal cavity of patients with gastric, colorectal, and pancreatic cancer.[10] It was shown that at the time of the operation, tumor cells occur with high frequency in the peritoneal cavity and in the bone marrow. However, it remains unclear if single tumor cells are of prognostic significance and have the ability to form metastatic disease.

No comprehensive immunocytologic studies exist concerning the prognostic significance of isolated intraperitoneal tumor cells. Therefore, we extended our former study

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to a larger collection of patients and investigated peritoneal lavage samples collected at the beginning of the operation. In median, the follow-up was 4 years. We also examined the bone marrow for micrometastatic cells because this is an easily accessible filter compartment of the bloodstream, and we compared the results of these two compartments of the body.

Immunocytologic studies of bone marrow samples from cancer patients used anticytokeratin antibodies, which stain epithelial cells not present in bone marrow.[4,5,9] This is not practicable for peritoneal cavity samples because of cross-reaction with mesothelial cells. We applied and compared a panel of different monoclonal antibodies (anti-CEA, anti-CA19-9, anti-Ra96, anti-C54-0, anti-17-1A) directed against tumor-associated antigens that have been tested in a control group and do not react with normal peritoneal and bone marrow cells. Bone marrow samples were also studied with the anticytokeratin antibody Kl-1.

We showed that tumor cells were frequently detectable in the peritoneal cavity and in the bone marrow. Their occurrence in the peritoneal cavity correlated to a highly significant degree with the postoperative survival rate of colorectal and gastric cancer patients.

MATERIAL AND METHODS

Patients

All patients were extensively informed and gave written consent for the investigations, including the bone marrow aspiration. The study was approved by the ethical commission of the University Hospital Kiel.

Eighty-four gastric and 109 colorectal patients with cancer who underwent surgery were investigated. No bone marrow sample was obtainable in 26 patients because they declined to give consent. Peritoneal lavage could not be performed in 22 patients because of adhesions.

A control group comprised 58 patients with a variety of nonmalignant diseases, including benign liver tumors (n = 10), sigmoid diverticulitis (n = 8), chronic pancreatitis (n = 7), cholecystolithiasis (n = 4), duodenal ulcers (n = 4), achalasia (n = 4), and hypersplenism (n = 4). Forty-five bone marrow and 43 peritoneal cavity samples were collected from the control group. Also, ascites from patients with liver cirrhosis (n = 5) and bone marrow samples from patients with benign hematologic diseases (n = 12) were investigated.

Samples

Bone marrow (8 mL) was aspirated from the right spina iliaca anterior at the beginning of the operation using a Jamshidi needle. Peritoneal lavage was performed before manipulation of the tumor. One liter of isotonic sodium chloride solution was instilled and immediately removed. The lavage solution was centrifuged (1200 g for 10 minutes). The cells were further processed by Ficoll-Paque (Pharmacia, Uppsala, Sweden) and were centrifuged onto microscopic slides (2.5×10^5 cells/slide). Cytospins were fixed in acetone and stored at −20 C.

Immunocytochemistry

Staining of cytospins was performed by the immunoperoxidase method with six different monoclonal antibodies, as described previously10: 1) C1P83[12]: anti-CEA; 2) CA19-9[13]: determinants of Lewis blood group antigens; 3) 17-1A[14]: membrane antigen; 4) Ra96[15]; mucin; 5) C54-0[16]: membrane antigen (because of a cross-reactivity with a subpopulation of lymphopoietic cells in the bone marrow, C-54-0 was used only in peritoneal cavity samples); and 6) Kl-1 (Dianova, Hamburg, Germany): cytokeratin (used only in bone marrow samples). Each antibody was tested on a slide with 2.5×10^5 cells. A positive control (WIDR–colon cancer cells) and a negative control (no specific monoclonal antibody) were stained in parallel to the samples. The microscopic evaluation was carried out independently by two investigators who were unaware of the patient data.

Evaluation of Data

Samples were evaluated as positive for tumor cells if at least one cell reacted with one of the monoclonal antibodies. The detection rate was correlated with the UICC classification of the tumor stage and the R classification.[17]

After surgery, patients were examined either in our outpatient clinic or by their general practitioner. Every 3 months a clinical examination and blood tests, including tumor markers CEA and CA19-9, were done, and every 6 months a sonography or CT scan and an endoscopy were performed.

Survival rates were determined by Kaplan-Meier test and calculations of significance by the log-rank test.

RESULTS

Control Group

In 43 of 45 patients, no cell staining was seen in bone marrow samples with Kl-1, C1P83, Ra96, CA19-9, and 17-1A. In 2 patients, single cells were stained with Kl-1, C1P83, and 17-1A. Also, 1 patient had positive cells for CA19-9. Both patients were strongly suspected of suffering from pancreatic cancer and were therefore treated by a Whipple operation. The histologic analysis could not confirm this diagnosis and found chronic pancreatitis.

The peritoneal lavage samples of patients with no malignant diseases showed, in 40 of 43 cases, no cross-reaction of the applied antibodies (C1P83, Ra96, CA19-9, 17-1A, C54-0). Two patients with chronic pancreatitis were positive for C54-0 or C1P83. A third patient with chronic hepatitis C was positive for C54-0.

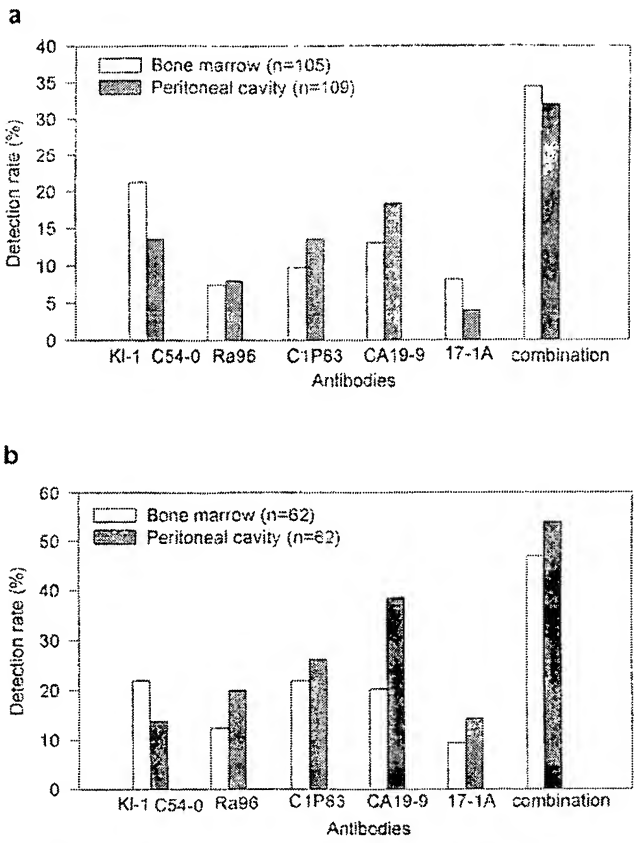

Figure 1. The percentage of positive samples for each monoclonal antibody and the combined evaluation in the bone marrow and the peritoneal cavity of (a) colorectal and (b) gastric cancer patients.

Colorectal Cancer

In 49% (53 of 109) of the patients, stained cells were detected in the bone marrow (33% [35/105]) or in the peritoneal cavity (31% [34/109]). Fourteen patients showed positive staining in both compartments.

Kl-1 showed the highest detection rate in bone marrow samples, followed by CA19-9 and C1P83 (anti-CEA). In peritoneal cavity samples, CA19-9 and C1P83 reacted with the highest frequency with tumor cells. The combination of all antibodies significantly increased the detection rate (Fig. 1A).

In bone marrow and peritoneal cavity samples, the detection rate increased in parallel with the tumor stage (Fig. 2). Interestingly, already 19% of patients with a stage I tumor had positive cells within the peritoneal cavity.

Forty-four percent of R0-resected patients (n = 96) showed tumor cell spread either in the peritoneal cavity (22% [21/94]) or in the bone marrow (33% [27/83]).

The postoperative 4-year survival rate was determined in 109 patients. The cumulative survival rates for peritoneal cavity and bone marrow findings are shown in Figure 3. After 4 years, 28% of patients with positive immunocytologic findings in the peritoneal cavity were alive *versus* 60% of patients with negative findings ($p = 0.0079$). No correlation was found by evaluating the results of bone marrow samples.

To determine if the antibodies vary in their correlation with the survival rate, each single antibody result was evaluated separately. A highly significant correlation with the survival and peritoneal cavity findings was found for C1P83 ($p < 0.0001$) and CA19-9 ($p = 0.0002$). The findings with C54-0 ($p = 0.015$) and Ra96 ($p = 0.03$) were also statistically significant. Only 17-1A did not detect prognostically relevant cells ($p = 0.48$). In bone marrow samples, Ra96-positive patients showed a worse but statistically not significant ($p = 0.2$) prognosis: all 9 positive patients died within 3 years, compared to a 40% survival rate of negative patients. All other antibodies, including Kl-1, showed no prognostic significance (C1P83: $p = 0.7$; CA19-9: $p = 0.48$; 17-1A: $p = 0.9$; Kl-1: $p = 0.42$).

Consequently, further evaluation of peritoneal cavity samples excluded 17-1A findings. Additionally, the results from C54-0 were not taken into account because this antibody did not show prognostic significance in gastric cancer and reacted with normal cells of lavage samples from three cases in the control group.

Patients were evaluated according to their tumor stage to determine if the occurrence of isolated tumor cells has a prognostic significance independent from the UICC classification. Table 1 summarizes the results for each tumor stage. In all stages, patients with positive peritoneal cavity findings showed a worse survival rate than negative patients (stage I: 75% *vs.* 93%; stage II: 80% *vs.* 82%; stage III: 33% *vs.* 65%; stage IV: 8% *vs.* 27%).

The low number of cases hindered the Kaplan-Meier calculation for each stage. Therefore, patients with an "early tumor stage" (stages I and II, n = 56) and advanced

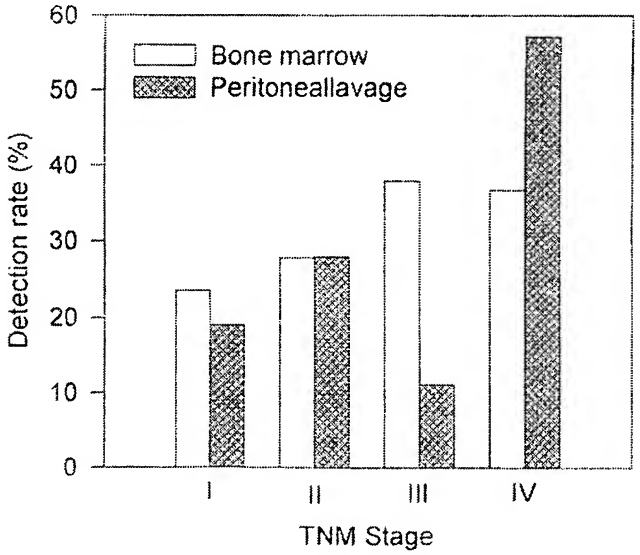

Figure 2. Correlation between the UICC tumor stage and positive findings in the bone marrow (bm) and peritoneal cavity (pc) of colorectal cancer patients. Bone marrow: stage I, n = 15; stage II, n = 33; stage III, n = 28; stage IV, n = 29. Peritoneal cavity: stage I, n = 21; stage II, n = 33; stage III, n = 27; stage IV, n = 28.

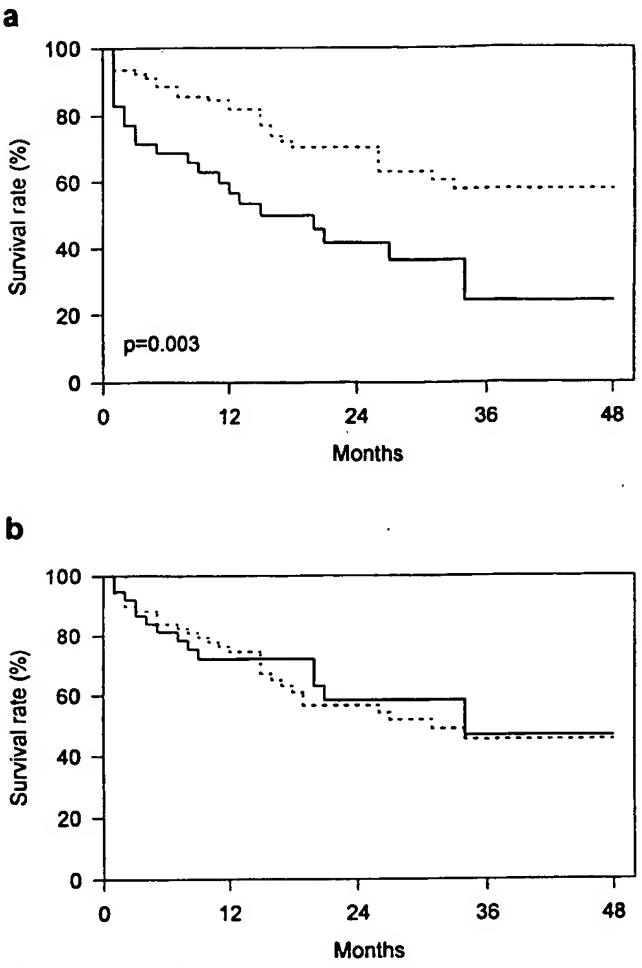

Figure 3. Kaplan-Meier calculation for the cumulative 4-year survival rate of patients with colorectal cancer with antibody-positive (Ab-positive) *versus* antibody-negative (Ab-negative) samples. (a) Results of peritoneal lavage samples ($p = 0.0079$). — Ab-positive ($n = 34$); - - - Ab-negative ($n = 75$) (b) Evaluation of bone marrow samples ($p = 0.9$). — Ab-positive ($n = 35$); - - - Ab-negative ($n = 70$)

Table 1. SURVIVAL OF COLORECTAL CANCER PATIENTS WITH POSITIVE/NEGATIVE FINDINGS IN THE PERITONEAL LAVAGE (PL) AND IN THE BONE MARROW (BM)

Stage	Positive PL	Negative PL	Positive BM	Negative BM
I	3/4 (75)	13/14 (93)	4/4 (100)	11/12 (92)
II	8/10 (80)	23/28 (82)	7/8 (87)	18/24 (75)
III	1/3 (33)	15/23 (65)	9/10 (90)	9/20 (45)
IV	1/13 (8)	3/11 (27)	0/9 (0)	5/17 (29)

Values are the numbers of patients that survived within the observation period in relation to the total number of cases with tumor stages I, II, III, and IV; the value in parentheses is the percentage of 4-year survival. (17-1A and C54-0 antibody results are excluded)

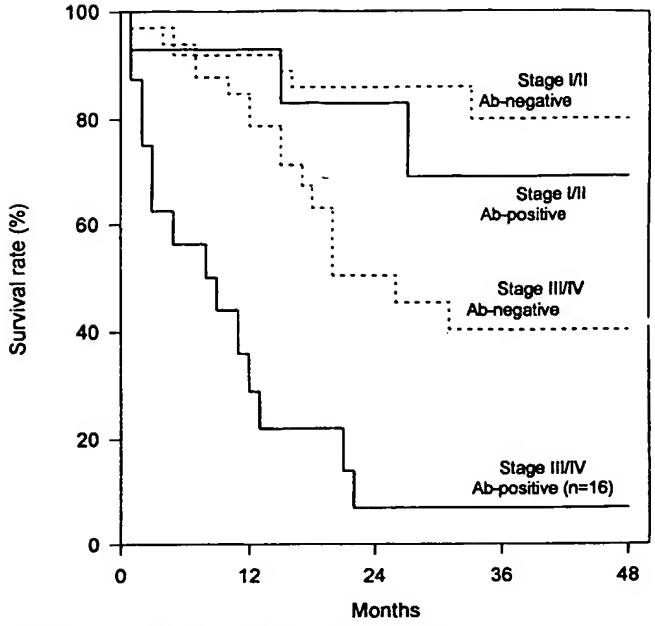

Figure 4. Correlation of the postoperative survival rate and immunocytologic findings in the peritoneal cavity of patients with colorectal cancer (17-1A and C54-0 antibody results are excluded). Cases are compared with early tumor (stages I and II) ($p = 0.48$) and advanced (stages III and IV) ($p < 0.0001$). Stage I/II Ab-negative ($n = 42$); Stage I/II Ab-positive ($n = 14$); Stage III/IV Ab-negative ($n = 34$)

cancer (stages III and IV, $n = 50$) were combined and evaluated with the log-rank test.

The survival rates of patients with stage I and II disease and a positive or negative peritoneal cavity finding ran parallel in the first 2 postoperative years. Afterward, the curves divided: after 3 years, 80% of patients with negative results were still alive *versus* 69% in the positive group ($p = 0.48$). In patients with stage III and IV disease, the difference between positive and negative findings became statistically highly significant ($p < 0.0001$) (Fig. 4). In bone marrow samples, no correlation was seen by evaluation of single tumor stages except in stage IV patients (see Table 1) and by Kaplan-Meier analysis of early and advanced patients (data not shown).

Gastric Cancer

Positive stained cells were detected in 64% (54 of 84) of the patients, with 53% (33 of 62) positive peritoneal cavity samples and 48% (30 of 62) positive bone marrow samples. Nineteen patients had disseminated tumor cells in both compartments. Figure 5 shows a typical microscopic picture of a C1P83-positive peritoneal cavity sample.

Kl-1 showed the highest detection rate in bone marrow samples, followed by C1P83 and CA19-9. In peritoneal cavity samples, C1P83 and CA19-9 stained tumor cells with the highest frequency. The combination of all antibodies significantly increased the detection rate (Fig. 1B).

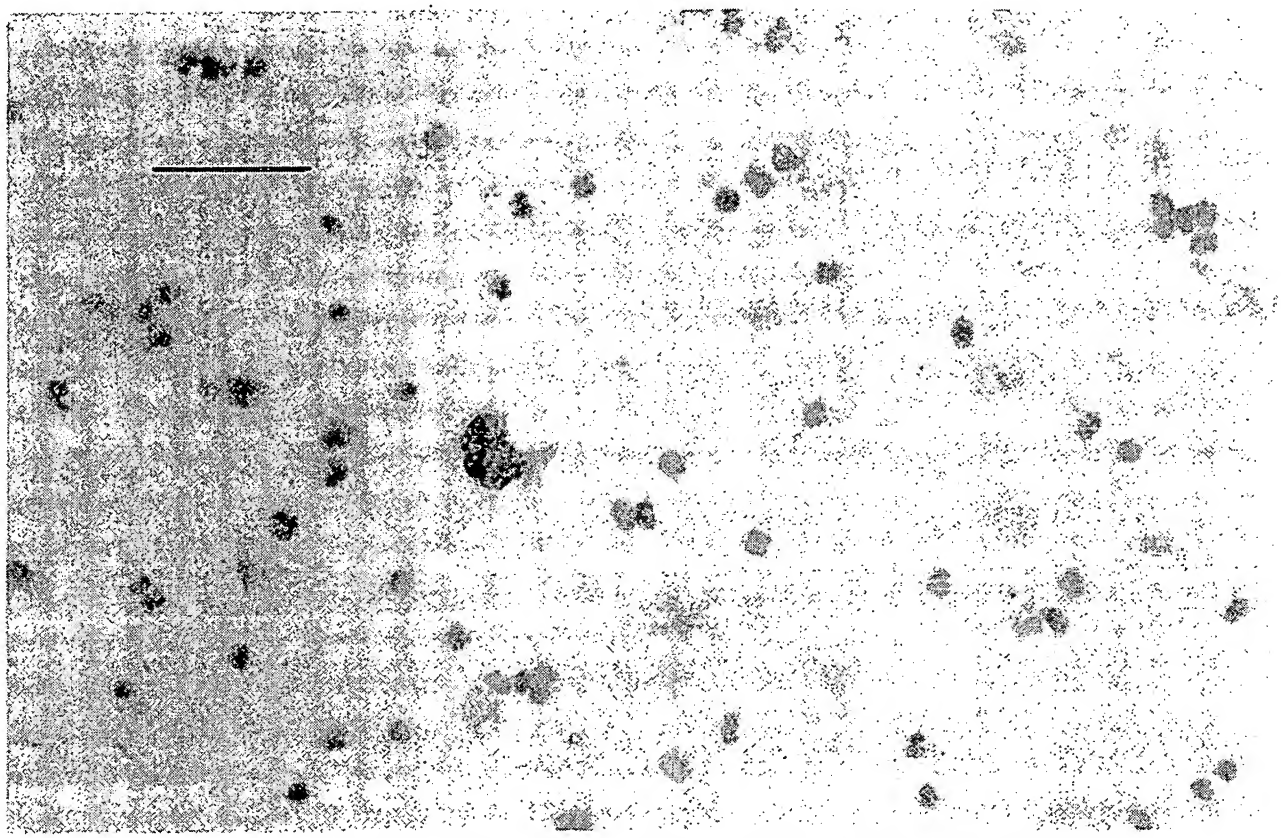

Figure 5. Cytospin of a peritoneal lavage sample from a patient with gastric cancer (stage II) stained with C1P83 (anti-CEA) antibody. Bar = 40 μm.

In peritoneal cavity samples, the detection rate increased in parallel with the tumor stage (Fig. 6), showing positive samples in 32% of stage I and 77% of stage IV patients.

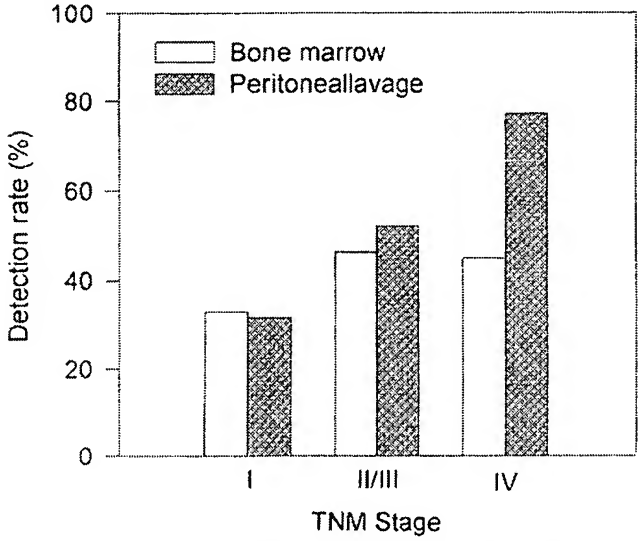

Figure 6. Correlation between the UICC tumor stage and positive findings in the bone marrow and peritoneal cavity of gastric cancer patients. Bone marrow: stage I, n = 20; stages II and III, n = 23 (stage II, n = 9; stage III, n = 14); stage IV, n = 19. Peritoneal cavity: stage I, n = 19; stages II and III, n = 21 (stage II, n = 10; stage III, n = 11); stage IV, n = 22.

Only a small increase was seen between bone marrow findings of progressing tumors.

R0-resected patients showed a tumor cell spread in 55% of the peritoneal lavage (n = 31) and in 49% of the bone marrow probes (n = 41).

The cumulative postoperative survival rate was correlated with the immunocytologic findings and showed highly significant results in peritoneal cavity samples ($p = 0.0038$), but no correlation was seen in bone marrow samples ($p = 1$) (Fig. 7).

The single antibody evaluation showed highly significant results for Ra96, CA19-9, and C1P83 ($p < 0.0001$) in peritoneal cavity samples. The 17-1A antibody also correlated ($p = 0.023$), but no correlation was found with C54-0 ($p = 0.9$). In bone marrow samples, C1P83 and KI-1 detected prognostically relevant cells. All 14 patients with C1P83 tumor cell staining died within 2 years ($p = 0.08$). In patients with advanced cancer, KI-1 detection of tumor cells correlated with a worse survival ($p = 0.12$).

Patients were evaluated according to tumor stage to determine if the occurrence of isolated tumor cells had a prognostic significance independent of the UICC classification. Results from C54-0 and 17-1A were excluded. Table 2 summarizes the results for each tumor stage. In all stages except stage I, patients with positive peritoneal findings showed a worse survival rate than negative patients (stage II: 50% *vs.* 100%; stage III: 40% *vs.* 56%;

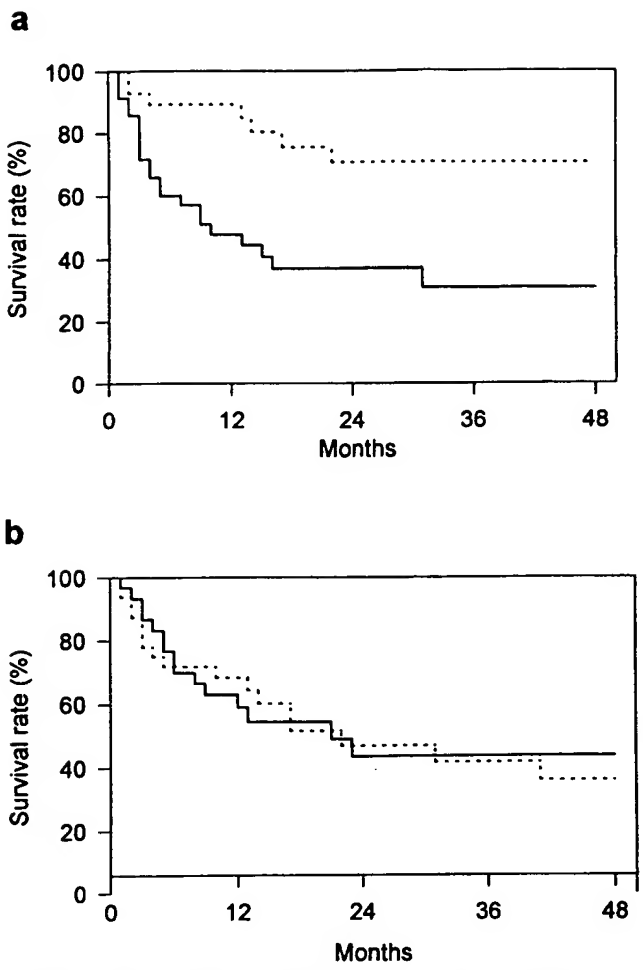

Figure 7. Kaplan-Meier calculation for the cumulative 4-year survival rate of gastric cancer patients with antibody-positive (Ab-positive) *versus* antibody-negative (Ab-negative) samples. (a) Results of peritoneal lavage samples (p = 0.0038). —— Ab-positive (n = 33); - - - Ab-negative (n = 29) (b) Evaluation of bone marrow samples (p = 1). —— Ab-positive (n = 30); - - - Ab-negative (n = 32)

stage IV: 7% *vs.* 50%). In stage I, the group size was too small to draw any conclusions (2 positive patients *vs.* 18 negative).

Kaplan-Meier curves were calculated with the log-rank test by combining stage I and II patients and stage III and IV patients. As in colorectal cancer, the survival curves in stage I and II patients ran parallel for 2 years before dividing. After 4 years, patients with negative peritoneal samples showed a significantly better survival rate than patients with positive findings (80% *vs.* 54%, p = 0.02). This difference was even more striking in advanced patients: The Kaplan-Meier calculation showed that all positive patients die within 2.5 years, but 54% of negative patients survive at least 4 years (p = 0.02) (see Fig. 8).

In bone marrow samples, patients with stage III and IV showed a worse survival when tumor cells were detected: at 4 years, 14% of stage III and 0% of stage IV positive patients survived *versus* 50% and 30% of negative patients, respectively. The combined evaluation of stage I and II and

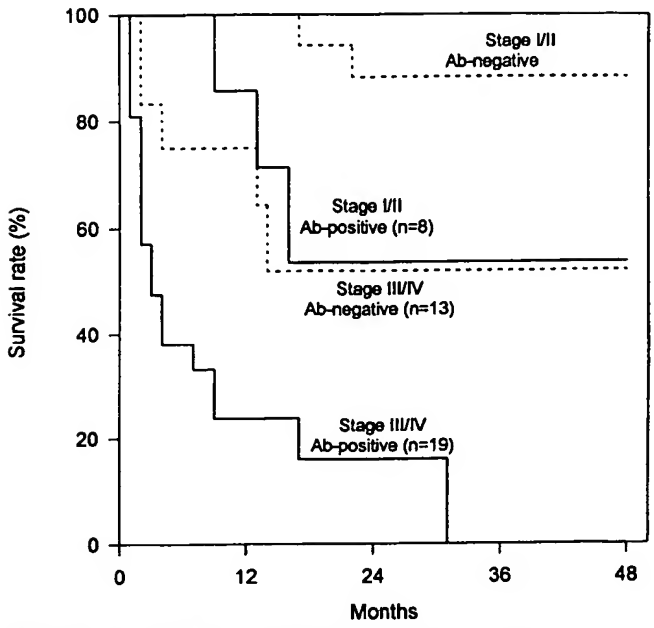

Figure 8. Correlation of the postoperative survival rate and immunocytologic findings in the peritoneal cavity of patients with gastric cancer (17-1A and C54-0 antibody results are excluded). Cases are compared with early tumor (stages I and II) (p = 0.02) and advanced (stages III and IV) (p = 0.02). Results with the antibodies 17-1A and C54-0 were excluded. Stage I/II, Ab-negative (n = 22)

stage III and IV patients, as performed by Kaplan-Meier analysis, showed no statistically significant differences in either group.

DISCUSSION

By immunocytologic techniques, it has become possible to detect isolated tumor cells in the bone marrow of various cancer patients. Most studies have been performed with breast cancer patients using an antibody specific for epithe-

Table 2. SURVIVAL OF GASTRIC CANCER PATIENTS WITH POSITIVE/NEGATIVE IMMUNOCYTOLOGICAL FINDINGS IN THE PERITONEAL LAVAGE (PL) AND IN THE BONE MARROW (BM)

Stage	Positive PL	Negative PL	Positive BM	Negative BM
I	2/2 (100)	15/18 (83)	7/7 (100)	6/9 (67)
II	3/6 (50)	4/4 (100)	4/4 (100)	2/3 (67)
III	2/5 (40)	5/9 (56)	1/7 (14)	5/10 (50)
IV	1/14 (7)	2/4 (50)	0/8 (0)	3/10 (30)

Values are the numbers of patients that survived within the observation period in relation to the total number of cases with tumor stages I, II, III, and the IV; value in parentheses is the percentage of 4-year survival. (17-1A and C54-0 antibody results are excluded)

lial cells to detect tumor cell spread in the bone marrow at the time of operation.[18] A strong correlation between tumor cell detection and survival could be seen; hence, in these patients, the finding of isolated cancer cells may serve as a new prognostic marker.[4] Further studies were published describing a similar approach to search for isolated tumor cells in the bone marrow of patients with lung cancer,[5] prostatic cancer,[7] and neuroblastoma.[6]

In contrast to these malignancies, bone metastases are rare in gastric and colorectal cancer. However, peritoneal carcinosis or metastases within the peritoneal cavity (e.g., in the liver and lymph nodes) occur in 90% of the patients.[11] Therefore, we investigated peritoneal cavity samples from colorectal and gastric cancer patients by an immunocytologic approach using a panel of five antibodies that react with several tumor-associated antigens. We also investigated the bone marrow because it might serve as a filtering system of the bloodstream, so circulating tumor cells could become detectable due to a cumulative effect.[9]

Previously,[10] we showed that our approach allows highly specific tumor cell detection in the bone marrow and in the peritoneal cavity. Despite the theoretical risk of nonspecific staining, we found no positive cells in the samples from the control group. In this study, we confirmed the practicability of our definition of tumor cell positivity (the finding of only one stained cell with one monoclonal antibody). The enlarged control group contained only two positive bone marrow and three positive peritoneal lavage samples. Two patients were treated by a Whipple operation because of strong suspicion of pancreatic cancer. A third patient suffered from chronic hepatitis C and liver cirrhosis and showed positive lavage cells for C54-0. Especially in peritoneal lavage samples, there is a theoretical risk of nonspecific staining of mesothelial cells in the peritoneal cavity.[19] Therefore, we propose excluding C54-0 from peritoneal lavage examination. However, it will be interesting to follow up the three patients with positive samples because of the remaining uncertainty about the diagnosis.

In our study, we found disseminated tumor cells in the bone marrow and the peritoneal cavity with similar frequency. Even in stage I, when direct tumor access can be completely excluded, about 22% of colorectal and 30% of gastric cancer patients had circulating tumor cells within the peritoneal cavity. This observation supports data from a cytologic study in gastric cancer.[20] In this study 3% of stage I gastric cancer patients had viable tumor cells within the peritoneal cavity; they most likely reached the peritoneum by pores and lymph vessels. In accordance with the significantly higher sensitivity of the immunocytologic method,[3] we achieved higher detection rates. Therefore, we found strong evidence that tumor cell spread is a general feature of gastrointestinal cancers and must be classified in most patients as generalized disease.

Whether isolated tumor cells can form metastatic disease and are therefore of prognostic significance remains to be elucidated. In our study, colorectal cancer patients whose bone marrow samples showed tumor cells—stained with Ra96 and C1P83 (anti-CEA)—had a worse prognosis. The anticytokeratin antibody Kl-1 detected a high-risk group of gastric cancer patients who suffered from stage III or IV disease. These results support to some extent data from Lindemann et al.[21] Using an anticytokeratin antibody, they found high numbers of tumor cells in the bone marrow of colorectal cancer patients and suggested that these cells had independent prognostic significance.

Our results strongly indicate that in gastrointestinal cancer, the investigation of peritoneal cavity cells is more relevant than the bone marrow approach. This finding is in accordance with the rarity of bone metastases but the high frequency of metastases within the peritoneal cavity.[11]

In colorectal and gastric cancers, the cumulative survival rates significantly correlated with the immunocytologic findings. Furthermore, patients with small tumors (stage I or II) and positive peritoneal cavity samples showed a worse prognosis; in gastric cancer, the difference was statistically significant ($p = 0.02$). In the advanced tumor stage (stages III and IV), all gastric cancer patients with positive staining are supposed to die within 2.5 years, but 54% of patients with negative staining survived. In colorectal cancer, all but 2 patients in the stage III and IV group with positive findings died, in contrast to a 40% 4-year survival rate in the negative group. Although no correlation between immunocytologic findings and the single tumor stage could be statistically calculated in most cases, striking differences could be seen in colorectal cancer stage I and IV patients. In stage I, 1 of 4 positive patients but only 1 of 14 negative patients died within 4 years. In stage IV, almost all positive patients (12 of 13) died within 2 years, but 27% of the 11 negative patients survived 4 years.

These results strongly suggest that the immunocytologic staining of peritoneal lavage samples serves as a new prognostic marker. A recently published method allows much more rapid immunostaining of peritoneal cavity samples by reducing the antibody incubation time with a microwave irradiation of the cells.[22] By using such a technique, it might be possible to get a diagnostic result while the operation is still in progress. This could help to guide surgical and adjuvant therapy. Stage IV patients with a negative peritoneal lavage might benefit from a radical surgical approach to achieve a R0 resection, but stage IV patients with a positive lavage most likely would not benefit from this approach. In gastric cancer patients, intraoperative prophylactic application of carbon-adsorbed mitomycin was shown to reduce the rate of tumor relapse.[1] This treatment could be helpful in lavage-positive patients with early tumor stages. Furthermore, it might be useful to offer adjuvant therapy in general to patients with positive peritoneal findings and a tumor stage I or II. Currently, these patients are excluded from adjuvant treatment because of the overall low risk of cancer relapse.[2]

It is unclear why the prognostic significance of bone marrow cells is of lower value. Perhaps the contact of tumor cells with peritoneal cells supports their ability to develop the full meta-

static phenotype, a hypothesis supported by the clinical observation of a high peritoneal carcinosis rate in colorectal and especially gastric cancer.[11] Cells in the bone marrow may be in the "wrong" environment and be kept in a dormant state, as data from Pantel et al.[23] suggest. Further studies will focus on characterizing the isolated cancer cells to elucidate local factors that may be important in the progress to metastatic disease.

The present immunocytologic approach has the disadvantage of being time-consuming and dependent on the skill of the investigator. Thus, from a practical point of view, this approach is unsuitable as a widely used routine method. Therefore, the nested reverse transcriptase–polymerase chain reaction (RT-PCR) has been used to detect disseminated tumor cells. Recently, it was shown that a nested RT-PCR with cytokeratin CK20 enables specific detection of tumor cells in the bone marrow of patients with gastrointestinal cancer.[24] This approach is not practicable in the peritoneal cavity. The nested RT-PCR with CEA, which has already been shown to detect gastrointestinal cancer cells in bone marrow samples,[25] might also be suitable for peritoneal lavage samples. Although nested RT-PCR is a useful tool, its reliability remains to be proven in the diagnosis of gastrointestinal cancer dissemination. The standardization of this extremely sensitive technique is one of the main obstacles and is currently being investigated.[26]

In summary, by using an immunocytologic approach, we showed for the first time that a minimal residual disease often becomes detectable in the peritoneal cavity of patients with colorectal and gastric cancer. The occurrence of isolated tumor cells could be correlated with a worse prognosis, even in early cancer stages. Therefore, the detection of these cells can serve as a new prognostic marker and will help to guide adjuvant therapy.

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References

1. Hagiwara A, Takahashi T, Kojima O, et al. Prophylaxis with carbon-adsorbed mitomycin against peritoneal recurrence of gastric cancer. Lancet 1992;339:629–631.
2. Moertel CG, Fleming TR, MacDonald JS, et al. Levamisole and fluorouracil for adjuvant therapy of resected colon carcinoma. N Engl J Med 1990;322:352–358.
3. Molino A, Colombatti M, Bonetti F, et al. A comparative analysis of three different techniques for the detection of breast cancer cells in bone marrow. Cancer 1991;67:1033–1036.
4. Diel I, Kaufmann M, Goerner R, et al. Detection of tumor cells in bone marrow of patients with primary breast cancer: a prognostic factor for distant metastasis. J Clin Oncol 1992;10:1534–1539.
5. Pantel K, Izbicki J, Passlick B, et al. Frequency and prognostic significance of isolated tumour cells in bone marrow of patients with non-small-cell lung cancer without overt metastases. Lancet 1996;347: 649–653.
6. Combaret V, Favrot MC, Kremens B, et al. Immunological detection of neuroblastoma cells in bone marrow harvested for autologous transplantation. Br J Cancer 1989;59:844–847.
7. Riesenberg R, Oberneder R, Kriegmair M, et al. Immunocytological double staining of cytokeratin and prostate specific antigen in individual prostatic tumor cells. Histochemistry 1993;99:61–66.
8. Schlimok G, Funke I, Pantel K, et al. Micrometastatic tumor cells in bone marrow of patients with gastric cancer: methodological aspects of detection and prognostic significance. Eur J Cancer 1991;27(11): 1461–1465.
9. Schlimok G, Funke I, Holzmann B, et al. Micrometastatic cancer cells in bone marrow: *in vitro* detection with anti-cytokeratin and in vivo labeling with anti-17-1A monoclonal antibodies. Proc Natl Acad Sci 1987;84:8672–8676.
10. Juhl H, Stritzel M, Wroblewski A, et al. Immunocytological detection of micrometastatic cells: comparative evaluation of findings in the peritoneal cavity and in the bone marrow of gastric, colorectal and pancreatic cancer patients. Int J Cancer 1994;57:330–335.
11. Doerr W, Seifert G, Uehlinger E. Spezielle Pathologische Anatomie, 2d ed. Berlin, Heidelberg, New York: Springer; 1973.
12. Hammarstrom S, Shively JE, Paxton RJ, et al. Antigenetic sites in carcinoembryonic antigen. Cancer Res 1989;49:4852–4858.
13. Koprowski H, Steplewski Z, Mitchell K, et al. Colorectal carcinoma antigens detected by hybridoma antibodies. Somatic Cell Genet 1979; 5:957–972.
14. Herlyn M, Steplewski Z, Herlyn D, Koprowski H. Colorectal carcinoma-specific antigen: detection by means of monoclonal antibodies. Proc Natl Acad Sci USA 1979;76:1438–1442.
15. Kalthoff H, Holl K, Schmiegel W, et al. A new mucin reacting monoclonal antibody for serum diagnosis and radioimmunoscintigraphy of pancreatic cancer. J Tumormarker Oncol 1987;2:75.
16. Schmiegel WH, Kalthoff H, Arndt R, et al. Monoclonal antibody-defined human pancreatic cancer-associated antigens. Cancer Res 1985;45:1402–1407.
17. Hermanek P, Scheibe O, Spiessl B, Wagner G. UICC TNM-Klassifikation Maligner Tumoren, 4th ed. Berlin, Heidelberg, New York, London, Paris, Tokyo: Springer; 1987.
18. Redding WH, Monoghan P, Imrie SF, et al. Detection of micrometastases in patients with primary breast cancer. Lancet 1983;2:1271–1274.
19. Luettges J, Neumann K, Pflyger K-H, Schmitz-Moormann P. Differentialzytologie von Erguβflyssigkeiten unter Anwendung von monoklonalen Antikörpern. Pathologe 1988;9:137–142.
20. Nakajima T, Harashima S, Hirata M, Kajitani T. Prognostic and therapeutic values of peritoneal cytology in gastric cancer. Acta Cytologica 1978;22(4):225–229.
21. Lindemann F, Schlimok G, Dirschedel P, et al. Prognostic significance of micrometastatic tumour cells in bone marrow of colorectal cancer patients. Lancet 1992;340:685–689.
22. Nomoto S, Nakao A, Takeuchi Y, et al. Intraoperative peritoneal washing cytology with the rapid immunoperoxidase method using microwave irradiation. J Surg Oncol 1995;60:30–34.
23. Pantel K, Schlimok G, Braun S et al. Differential expression of proliferation-associated molecules in individual micrometastatic carcinoma cells. J Natl Cancer Inst 1993;85:1419–1424.
24. Soeth E, Roeder C, Juhl H, et al. The detection of disseminated tumor cells in bone marrow from colorectal cancer patients by a cytokeratin-20-specific nested reverse transcriptase–polymerase chain reaction is related to the stage of disease. Int J Cancer (Pred Oncol) 1996;69: 278–282.
25. Gerhard M, Juhl H, Kalthoff H, et al. Specific detection of carcinoembryonic antigen-expressing tumor cells in bone marrow aspirates by polymerase chain reaction. J Clin Oncol 1994;12(4):725–729.
26. Jung R, Ahmad-Nejad P, Wimmer M, et al. Quality management and influential factors for the detection of single metastatic cancer cells by reverse transcriptase PCR. Eur J Clin Chem Clin Biochem 1997;35(1): 3–100.